

# Effects of a Europium-shift Reagent Upon the PMR Spectrum of Some Triglycerides

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In this journal, Almqvist *et al.* recently reported on the PMR spectral analysis (100 MHz) of almond oil dissolved in  $\text{CCl}_4$ , to which various quantities of  $\text{Eu}(\text{fod})_3$  were added.<sup>1</sup> Shift patterns were reported which they believed differed from those observed in our study (60 MHz) of a number of pure triglycerides in similar solutions.<sup>2</sup> The purpose of this communication is to clarify some of the apparent differences, to draw attention to the effect of some impurities, and to point out a weakness in the use of attenuation factors.

Fig. 1. contains data concerning the apparent shift of protons of *rac*-glycerol 1,2-dipalmitate 3-oleate (PPO). Shifts were measured relative to the internal standard, tetramethylsilane (TMS) proton resonance. The figure indicates that at low values of the  $\text{Eu}(\text{fod})_3$ -to-lipid ratio ( $X$ ), the curves rise sharply in approximately linear fashion. At higher values

of  $X$  the slopes decrease, and the proton resonances corresponding to the  $\beta$ - and  $\alpha$ -methylenes of the fatty acid moieties\* exhibit a coalescence in the case of the former and a crossover in the case of the latter.

Although Almqvist *et al.* report the same general shape for the curves in Fig. 1, a number of anomalies were observed. At low values of  $X$ , they observed little change in the apparent shift until  $X \approx 0.3$ . After this point their curves are similar to ours except for a slight dip reported by Almqvist in the region  $X \approx 2.3$ . Thus there is a report of at least one inflection point, although we have seen none in this study. Our data indicate a crossover of the  $\alpha$ -methylene peaks at  $X \approx 1.8$ , but the data of Almqvist *et al.* indicate that this occurs at  $X = 2.5$  and do not indicate a coalescence of  $\beta$ -methylenes even up to  $X \approx 5$ .

An important difference in the two studies is reflected in our use of pure model compounds compared to the use of an oil which may have contained traces of free acids. The previous authors have indicated that their assignments of  $X$  values might have been slightly high due to this. This is an attractive possibility since

$$(1/X_{\text{true}}) = (1/X_{\text{apparent}}) + [\text{mmol free acid/mmole Eu}(\text{fod})_3]$$

which would explain the disagreement in  $X$  for the phenomena observed. In an attempt to investigate this further, solutions of a triglyceride in which 10% of the lipid was oleic acid were studied by PMR after successive incremental additions of  $\text{Eu}(\text{fod})_3$ . The shift data from these experiments substantiated the predictions and also helped to explain the relatively flat curve at low  $X$  values observed by Almqvist. The resonances associated with the carboxylic acid were imperceptible at these low concentrations. Acid interacts with the shift reagent to a far greater degree than does the triglyceride, resulting in little or no shift of triglyceride resonances until an  $X$  value was reached at which virtually all of the acid had been complexed by  $\text{Eu}(\text{fod})_3$ .

It has been our experience that  $\text{Eu}(\text{fod})_3$  purchased from suppliers is somewhat

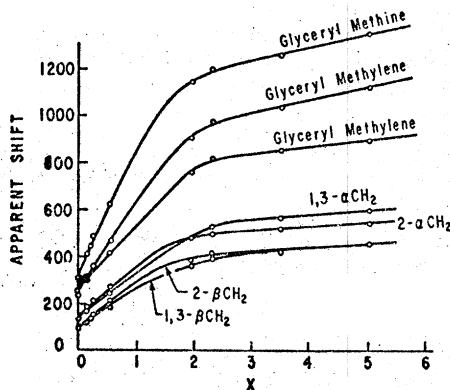


Fig. 1. Apparent chemical shifts (Hz) for PPO in  $\text{CCl}_4$  containing various concentrations of  $\text{Eu}(\text{fod})_3$ .  $X$  is the  $\text{Eu}(\text{fod})_3$ /triglyceride molar ratio. Spectrum obtained at 60 MHz using TMS as internal standard.

\* We refer to  $\alpha$ ,  $\beta$ , etc., as the methylene groups along the fatty acid chain, and 1,2,3 as positions on the glycerol moiety.

variable in properties due probably to contamination by Lewis bases such as water.<sup>3</sup> Some of the materials have been rejected on the basis of depressed transition temperatures<sup>4</sup> determined by differential scanning calorimetry. In other cases, spurious unidentified peaks in the PMR spectra of solutions of the reagents have been observed which led to our rejection of the  $\text{Eu}(\text{fod})_3$ . With samples of  $\text{Eu}(\text{fod})_3$  which have passed these tests, minor differences in apparent shift at, for example, high values of  $X$  have been observed.

In our previous work assignment of  $\alpha$ -methylene resonances was made primarily after consideration of the relative peak areas. In order to dispel any doubt concerning these assignments,  $\alpha$ -deuteriated oleic acid was prepared and used for the synthesis of selectively deuteriated POP ( $\text{PO}_{\alpha\text{D}_2}\text{P}$ ). Fig. 2a demonstrates the absence of the upfield ( $\delta$  8.2)  $\alpha$ -methylene resonance (except for a resonance corresponding to a small amount of proteo isomer). Furthermore,  $\text{Eu}(\text{fod})_3$ -complexed *rac*-glycerol 1,2-distearate 3-oleate (SSO)

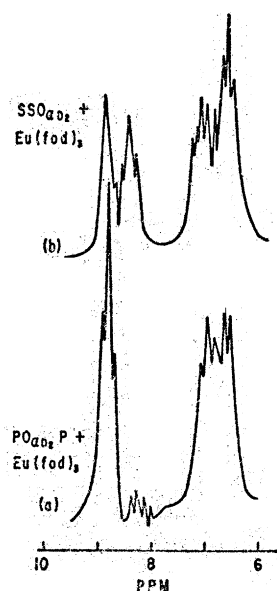


Fig. 2. (a.) PMR spectrum of the  $\alpha$ - and  $\beta$ -methylene protons of  $\text{PO}_{\alpha\text{D}_2}\text{P}$  in  $\text{CCl}_4$  containing  $\text{Eu}(\text{fod})_3$  (60 MHz).  $X \approx 2.4$ . (b.) PMR spectrum of the  $\delta$ - and  $\beta$ -methylene protons of  $\text{SSO}_{\alpha\text{D}_2}$  in  $\text{CCl}_4$  containing  $\text{Eu}(\text{fod})_3$  (60 MHz).  $X \approx 2.4$ .

was demonstrated to display spectral characteristics similar to PPO. A sample of  $\text{SSO}_{\alpha\text{D}_2}$  was examined under conditions similar to those employed in the study of  $\text{PO}_{\alpha\text{D}_2}\text{P}$ , and the absence of the originally observed downfield triplet, of the composite quartet ( $\delta$  9.1) was noted (Fig. 2b).

In view of this evidence, there can be no doubt about our initial assignment of resonances corresponding to the  $\alpha$ -methylene protons of the triglycerides. The curve denoted as 1,3- $\alpha\text{CH}_2$  in Fig. 1 represents the mean-apparent-shift value of these resonances for the dissymmetric molecular species PPO, and is nearly coincident with the center of gravity of the  $\alpha$ -methylene resonances of symmetric species such as POP.

Several authors have used attenuation factors<sup>5</sup> or induced shift ratios<sup>1,6</sup> to describe the chemical shift phenomenon. We wish to point out that such attenuation factors should be used with great care, especially in cases where "resonance crossovers" are demonstrated (Fig. 1). Since two protons may appear at an identical chemical shift, separate, and then cross over at higher  $X$ , attenuation factors may be variable and hence misleading.

In conclusion, we would like to emphasize that  $\text{Eu}(\text{fod})_3$  can provide important information concerning the structure of triglycerides. However, care should be exercised when relying upon the calculated ratios of  $\text{Eu}(\text{fod})_3$ /lipid and the chemical shift which results from these ratios. It can be particularly misleading to use these values when studying complex mixtures which, as we have shown, can contain spectroscopically undetectable complexible materials.

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